

RESEARCH ARTICLE

Comparative Evaluation of Sol–Gel, Hydrothermal, and Precipitation–Synthesized Tricalcium Silicate (Ca_3SiO_5) for Dual Osteogenic and Antibacterial Applications

A. Z. Sayyed¹, A. M. Patil^{1,*}, J. P. Sonawane²

ABSTRACT: Calcium silicates have emerged as promising bioactive materials for bone tissue engineering due to their excellent biocompatibility, osteoconductivity, and antibacterial properties. However, optimizing synthesis methods to maximize both osteogenic potential and infection control remains a significant challenge. This study comparatively evaluated the physicochemical and biological performance of tricalcium silicate (Ca_3SiO_5) synthesized via three distinct methods: chemical precipitation (CS-Precip), hydrothermal (CS-Hydro), and sol-gel (CS-Sol-gel). The synthesized materials were characterized using X-ray diffraction (XRD), Raman spectroscopy, and scanning electron microscopy (SEM) to analyze structural, phase, and morphological properties. Crystallite sizes were determined to be ~10.80 nm, 9.72 nm, and 9.81 nm for sol-gel, hydrothermal, and precipitation methods, respectively. Osteogenic potential was assessed using MG-63 osteoblast-like cells, while antibacterial efficacy was evaluated against *Staphylococcus aureus* and *Escherichia coli*. The sol-gel derived Ca_3SiO_5 demonstrated superior biological performance, exhibiting the highest alkaline phosphatase (ALP) activity of approximately 135% relative to the control at 50 $\mu\text{g/mL}$, indicating enhanced osteoblast differentiation. Additionally, it produced the largest inhibition zones (~13 mm) against *S. aureus* and required the lowest minimum inhibitory concentration (MIC), confirming its potent antibacterial activity. The enhanced performance of sol-gel synthesized material is attributed to its controlled phase purity, lower lattice strain, and favorable ion release profile, particularly higher Si^{4+} release. These findings establish sol-gel synthesized Ca_3SiO_5 as a highly promising candidate for bone defect repair applications requiring both regenerative and antimicrobial functionality.

Keywords: Tricalcium Silicate, Sol-Gel Synthesis, Osteogenic Activity, Antibacterial Properties, Bone Tissue Engineering, Bioactive Ceramics

Received: 25 January 2026; Revised: 20 April 2026; Accepted: 11 May 2026; Published Online: 28 May 2026

1. INTRODUCTION

Bone defects arising from tumor resections, traumatic injuries, congenital abnormalities, or degenerative skeletal conditions represent a substantial clinical burden, often leading to significant functional impairment and diminished quality of life for affected individuals [1, 2]. The inherent complexity of bone tissue, combined with the intricate biological processes required for its regeneration, has

¹ Department of Physics, R. C. Patel Arts Commerce and Science College, K.B.C. North Maharashtra University, Shirpur-425405, India

² Department of Chemistry, R. C. Patel Arts Commerce and Science College, K.B.C. North Maharashtra University, Shirpur-425405, India

* Author to whom correspondence should be addressed:
ampatil67@gmail.com (A.M. Patil)

positioned the restoration of bone structure and function as one of the most formidable challenges in modern regenerative medicine. Traditional approaches such as autografts and allografts have been widely employed, yet they carry inherent limitations including donor site morbidity, limited availability, immune rejection risks, and potential disease transmission [3]. These constraints have catalyzed extensive research into synthetic biomaterials that can effectively bridge bone defects while promoting natural healing processes.

Among the various synthetic materials explored for bone tissue engineering, calcium silicate-based ceramics have garnered considerable attention owing to their exceptional biocompatibility, bioactivity, and cost-effectiveness [4, 5]. These materials exhibit the remarkable ability to bond directly with living bone tissue through the formation of a biologically active hydroxyapatite layer upon exposure to physiological fluids. Furthermore, calcium silicates possess a unique property that distinguishes them from many other bioceramics, they create a localized alkaline environment during degradation, which not only facilitates bone regeneration but also imparts inherent antibacterial activity [6, 7]. This dual functionality addresses two critical requirements in bone defect management: promoting osteogenesis while simultaneously preventing bacterial colonization and subsequent infection, which remains a leading cause of implant failure and surgical complications [8].

The escalating threat of antibiotic-resistant bacterial infections has intensified the search for biomaterials that can provide intrinsic antimicrobial protection without relying on conventional antibiotics [9, 10]. Bone tissue engineering faces additional complexities, including the challenge of achieving rapid vascular ingrowth to sustain newly formed tissue, maintaining mechanical integrity during the healing process, and ensuring successful integration with surrounding host tissue [1, 11]. The pioneering work of Hench and colleagues in the 1970s, who developed bioactive glass compositions based on $\text{SiO}_2\text{-Na}_2\text{O-P}_2\text{O}_5\text{-CaO}$ systems, established the foundation for understanding how inorganic materials can actively participate in biological processes [12]. Subsequent research has demonstrated that the inorganic component of biomaterials significantly influences their bioactivity, with calcium silicate systems emerging as particularly promising candidates due to their ability to stimulate cellular responses and promote osteogenic differentiation [13–15].

Tricalcium silicate (Ca_3SiO_5), a principal component of Portland cement, has emerged as a material of significant interest for biomedical applications due to its excellent bioactive properties. However, the synthesis method employed profoundly influences the physicochemical characteristics of the resulting material, including crystallinity, particle morphology, specific surface area, and ion release kinetics. Various synthesis techniques including hydrothermal, sol-gel, co-precipitation, carbonation, and spray pyrolysis have been explored for producing calcium silicate-based materials [16–19]. While these methods offer

distinct advantages in terms of cost, scalability, and process control, the relationship between synthesis parameters and biological performance remains incompletely understood. The selection of an optimal synthesis approach is therefore critical, as it directly impacts the material's osteogenic potential and antibacterial efficacy.

Despite the growing body of research on calcium silicate-based biomaterials, systematic comparative studies evaluating how different synthesis methodologies influence their performance for dual osteogenic and antibacterial applications are notably scarce. The novelty of this work lies in its comprehensive, side-by-side comparison of three cost-effective synthesis techniques, sol-gel, hydrothermal, and co-precipitation, specifically tailored for producing Ca_3SiO_5 with optimized biological properties. While individual synthesis routes have been previously reported, this study provides a unique perspective by correlating specific structural features, including crystallite size, lattice strain, and phase purity, with biological outcomes, thereby establishing clear structure-activity relationships that can guide future material design.

In this paper, we report the synthesis of tricalcium silicate via sol-gel, hydrothermal, and co-precipitation methods, followed by systematic characterization using X-ray diffraction, Raman spectroscopy, and scanning electron microscopy to elucidate structural, phase, and morphological differences arising from each synthesis route. The biological performance of the synthesized materials was comprehensively evaluated through in vitro studies, including assessment of cytocompatibility using MG-63 osteoblast-like cells, alkaline phosphatase activity as an indicator of osteogenic differentiation, and antibacterial efficacy against clinically relevant pathogens including *Staphylococcus aureus* and *Escherichia coli*. By establishing correlations between synthesis-dependent material properties and biological responses, this investigation aims to identify the most promising synthesis strategy for developing Ca_3SiO_5 -based biomaterials with enhanced dual functionality for bone tissue engineering applications.

2. EXPERIMENTAL DETAILS

2.1. Materials

All chemical reagents employed in this study were of analytical grade and used without further purification. Calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\geq 99\%$), tetraethyl orthosilicate (TEOS, $\geq 99\%$), sodium metasilicate nonahydrate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$), ethanol ($\geq 99.8\%$), and ammonia solution (NH_4OH , 25%) were procured from Loba Chemie. Deionized water (resistivity $18.2 \text{ M}\Omega \cdot \text{cm}$) was used throughout the synthesis procedures.

2.2. Synthesis of Tricalcium Silicate (Ca_3SiO_5)

2.2.1. Sol-Gel Synthesis Method

Tricalcium silicate was synthesized via a modified sol-gel approach as reported previously [20]. In a typical procedure, 10 mL of tetraethyl orthosilicate was dispersed in hydrolyzed ethanol solution prepared by mixing 1 mL of ethanol with 40 mL of deionized water under continuous magnetic stirring at ambient temperature for 30 minutes to facilitate complete hydrolysis. Subsequently, 4.72 g of calcium nitrate tetrahydrate was introduced into the sol, and the pH of the resulting mixture was adjusted to approximately 11 by dropwise addition of ammonia solution. The reaction mixture was maintained under constant stirring at room temperature for 24 hours to allow complete gelation and aging. The resulting gel was dried in a hot air oven at 60°C for 24 hours, followed by calcination at 800°C for 2 hours in a muffle furnace with a heating rate of 5°C min⁻¹. The calcined powder was washed repeatedly with distilled water to remove residual nitrates and subsequently dried at 60°C for 4 hours. The final product was collected and stored for further characterization.

2.2.2. Hydrothermal Synthesis Method

For hydrothermal synthesis, 9.44 g of calcium nitrate tetrahydrate was dissolved in 50 mL of deionized water, and 6.08 g of sodium metasilicate nonahydrate was separately dissolved in 50 mL of deionized water under vigorous stirring. Both solutions were mixed thoroughly, and the pH of the combined solution was adjusted to ~11 using NH_4OH solution. The resulting suspension was transferred into a Teflon-lined stainless-steel autoclave (capacity 150 mL) and subjected to hydrothermal treatment at 180°C for 24 hours. After completion of the reaction, the autoclave was allowed to cool naturally to room temperature. The precipitate was collected by centrifugation at 10,000 rpm for 10 minutes, washed several times with deionized water, and subsequently dried in an oven at 60°C for 4 hours. The dried powder was used for characterization without any additional purification steps.

2.2.3. Co-Precipitation Method

Tricalcium silicate was also prepared using a co-precipitation technique. In this process, 7.08 g of calcium nitrate tetrahydrate and 9.12 g of sodium metasilicate nonahydrate were dissolved separately in 100 mL of deionized water each. The two solutions were mixed under continuous stirring at room temperature, and the pH was maintained at ~11 by adding NH_4OH solution. The reaction mixture was stirred for 2 hours to ensure complete precipitation. The resulting precipitate was recovered by centrifugation, washed repeatedly with deionized water to remove by-products and excess reagents, and dried at 60°C for 24 hours. The dried powder was subsequently calcined at 800°C for 2 hours in a muffle furnace. The final product was collected and stored in

a desiccator for further characterization.

2.3. Characterization Techniques

The phase composition and crystal structure of the synthesized Ca_3SiO_5 powders were determined using X-ray diffraction (XRD) with a PANalytical X'Pert Pro diffractometer operating at 40 kV and 40 mA with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$). The diffraction patterns were recorded over a 2θ range of 10° to 60° with a step size of 0.02° and a scan rate of 2° min⁻¹. Phase identification was performed by comparing the obtained patterns with standard JCPDS database files. The crystallite size was calculated using the Scherrer equation from the highest intensity peak corresponding to the (230) plane, and lattice strain was determined from the peak broadening analysis.

Raman spectroscopic analysis was performed using a Horiba Jobin Yvon HR800 Raman spectrometer equipped with a 532 nm Nd:YAG laser source. The spectra were recorded in the wavenumber range of 150 to 1500 cm⁻¹ with a spectral resolution of 2 cm⁻¹. The laser power was maintained at 10 mW to avoid sample heating effects.

Surface morphology and particle size distribution of the synthesized materials were examined using a scanning electron microscope (SEM, JEOL JSM-7600F) operated at an accelerating voltage of 15 kV. Prior to imaging, the powder samples were mounted on aluminum stubs using conductive carbon tape and sputter-coated with a thin layer of gold to prevent charging effects.

The cumulative ion release profiles of Ca^{2+} and Si^{4+} ions from the synthesized materials were evaluated by immersing 0.1 g of each sample in 10 mL of phosphate-buffered saline (PBS, pH 7.4) at 37°C under static conditions. Supernatants were collected at predetermined time intervals of 1, 3, 7, 14, and 21 days, and the ion concentrations were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, PerkinElmer Optima 8000). All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1. X-ray Diffraction (XRD) Studies

The crystal structure and phase composition of the synthesized tricalcium silicate materials were investigated using X-ray diffraction analysis. The theoretical diffraction pattern of tricalcium silicate (Ca_3SiO_5) was simulated based on its triclinic polymorph structure with monoclinic space group symmetry, as shown in Figure 1(a). The crystallographic analysis confirmed the presence of isolated $[\text{SiO}_4]^{4-}$ orthosilicate tetrahedra, which is characteristic of nesosilicate mineral classification. The prominent diffraction peaks observed at 2θ values of 22.65° (021), 24.83° (131), 29.27° (132), 31.75° (122), 32.07° (230), 33.98° (212), 37.54° (240), 39.12° (312), 40.79° (331), 42.67° (400), 45.09° (303),

and 46.26° (152) correspond to the characteristic reflections of the Ca_3SiO_5 phase. The structural visualization generated using VESTA software (Figures 1b and 1c) clearly shows the polyhedral arrangement wherein calcium atoms (mint-green), silicon atoms (blue), and oxygen atoms (red) are positioned within distinct coordination polyhedra, consistent with the crystallographic data reported in PDF card 00-049-0442 [21, 22].

The experimental XRD patterns of Ca_3SiO_5 synthesized via sol-gel, hydrothermal, and co-precipitation methods are presented in Figures 2(a-c), respectively. All diffraction patterns were recorded using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) and exhibited excellent agreement with the theoretically simulated pattern, confirming the successful formation of the desired crystalline phase. However, notable differences were observed among the three synthesis routes. The co-precipitation method yielded an additional low-intensity peak at approximately 24° (2 θ), positioned between the (021) and (131) reflections, which corresponds to the presence of a minor Ca_2SiO_4 impurity phase. This observation suggests that the co-precipitation approach offers comparatively less control over phase selectivity during synthesis, whereas both sol-gel and hydrothermal methods produced phase-pure Ca_3SiO_5 without detectable secondary phases.

The crystallite size and lattice strain were quantitatively determined from the most intense diffraction peak corresponding to the (230) plane using the Scherrer equation [23]. The calculated crystallite sizes were 10.80 nm for sol-gel derived Ca_3SiO_5 , 9.72 nm for hydrothermally synthesized material, and 9.81 nm for the co-precipitation product. These values indicate that all three synthesis methods produce nanocrystalline materials with comparable crystallite dimensions, though the sol-gel route yielded slightly larger crystallites. The lattice strain values were determined to be 0.1119, 0.1324, and 0.1326 for sol-gel, hydrothermal, and co-precipitation synthesized materials, respectively.

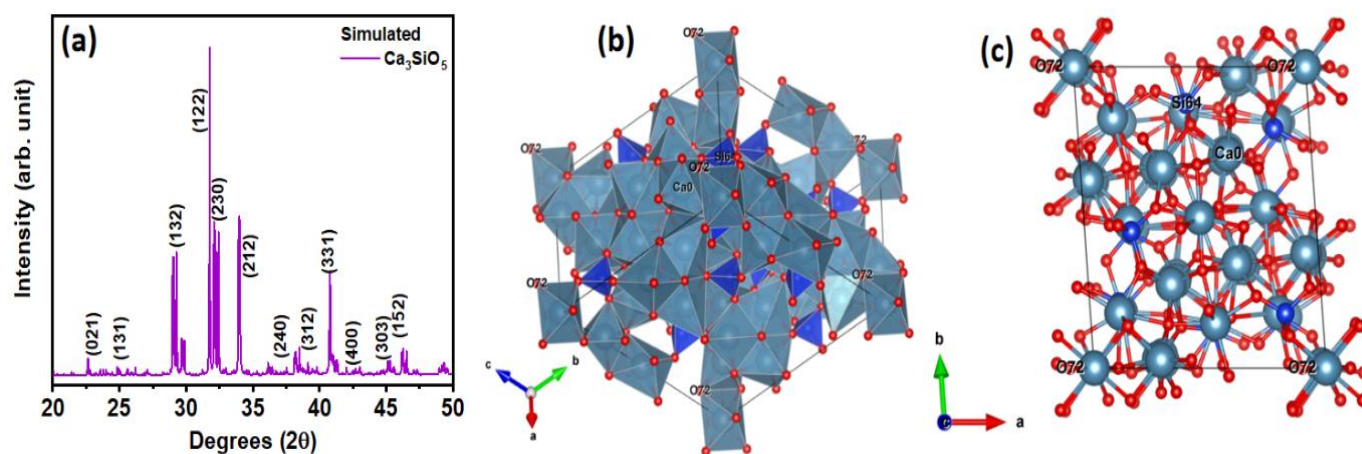


Fig. 1. (a) Simulated powder X-ray diffraction pattern of Ca_3SiO_5 , (b) Polyhedral visualization, (c) Structural visualization showing Si (blue), O (red), and Ca (mint-green).

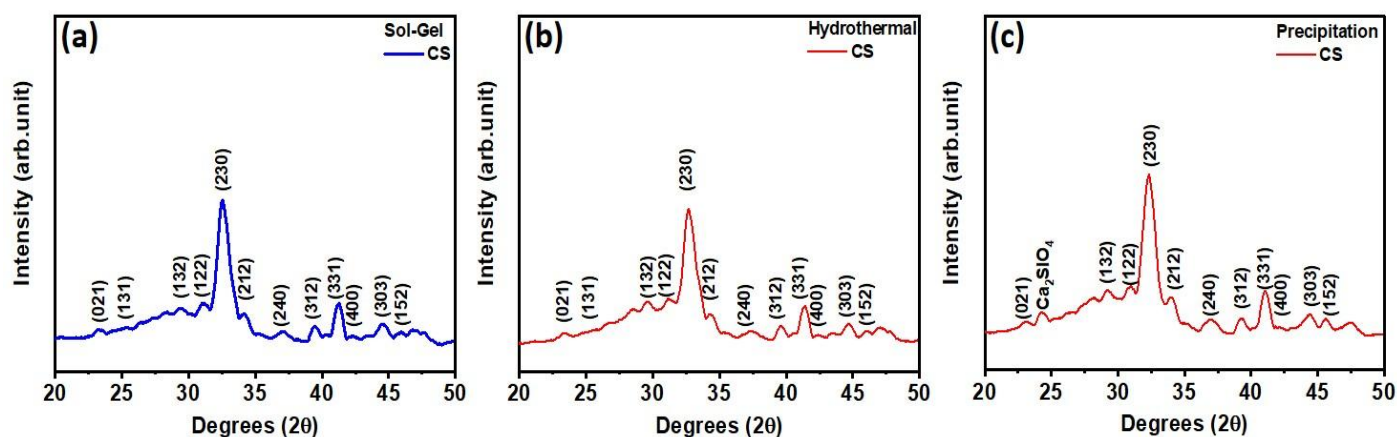


Fig. 2. X-ray diffraction spectra of Ca_3SiO_5 synthesized by (a) sol-gel, (b) hydrothermal, and (c) co-precipitation methods.

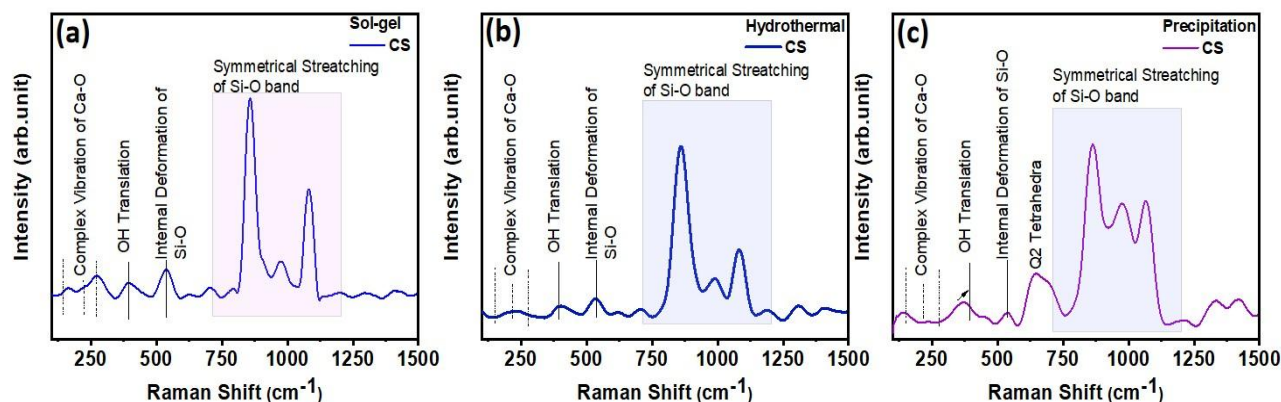


Fig. 3. Raman spectra of Ca_3SiO_5 synthesized by (a) sol-gel, (b) hydrothermal, and (c) co-precipitation methods.

The significantly lower lattice strain observed in the sol-gel derived sample suggests a more ordered crystal lattice with fewer structural defects. In contrast, the higher strain values observed for both hydrothermal and precipitation-derived materials indicate greater lattice distortion, likely arising from localized structural imperfections and non-uniform crystallite growth during synthesis. The presence of Ca_2SiO_4 impurity in the co-precipitation sample may also contribute to the observed strain due to lattice mismatch at phase boundaries. These findings highlight the superior structural quality achieved through the sol-gel approach, which provides better control over crystallization kinetics and phase purity.

3.2. Raman Spectroscopic Analysis

Raman spectroscopy was employed to provide complementary information regarding the molecular structure and phase composition of the synthesized materials. The Raman spectra of Ca_3SiO_5 prepared by sol-gel, hydrothermal, and co-precipitation methods are presented in Figures 3(a-c), recorded over the spectral range of 150 to 1500 cm^{-1} . The high-frequency region (800-1000 cm^{-1}) serves as a diagnostic fingerprint for silicate materials, corresponding to the symmetric stretching vibrations of Si-O bonds within the orthosilicate tetrahedra. The observed spectra in this region exhibit multiple well-resolved peaks, which arise from the non-equivalent crystallographic sites occupied by silicon atoms within the asymmetric unit cell of the triclinic structure.

The spectral region around 250 cm^{-1} shows distinct bands attributed to the complex vibrational modes involving Ca-O stretching interactions, confirming the presence of calcium-oxygen bonds in the crystal structure. Additionally, a band near 380 cm^{-1} was observed in all samples, which is characteristic of OH translation modes associated with surface hydration effects [24, 25]. Notably, the Raman spectrum of the co-precipitation synthesized sample (Figure 3c) exhibits an enhanced peak corresponding to Q_2 tetrahedra, which provides further evidence for the minor formation of

Ca_2SiO_4 phase. This observation is consistent with the XRD findings and confirms that the co-precipitation method produces a biphasic system with trace amounts of dicalcium silicate. The Raman spectra of sol-gel and hydrothermally synthesized materials showed no such additional features, corroborating their phase purity. The sharper and more defined peaks observed in the sol-gel derived sample suggest higher crystallinity and better structural ordering, which correlates well with the lower lattice strain values determined from XRD analysis.

3.3. Morphological Analysis

The surface morphology and particle characteristics of the synthesized Ca_3SiO_5 powders were examined using scanning electron microscopy. Figures 4(a) and 4(b) present SEM images of the sol-gel derived material at magnifications of $\times 5,000$ and $\times 20,000$, respectively. The micrographs reveal highly agglomerated particles with irregular morphology and a wide size distribution ranging from submicron to several micrometers. The agglomeration is a common feature observed in sol-gel derived ceramic powders, arising from the strong interparticle interactions during gelation and subsequent drying processes.

The morphological features of hydrothermally and co-precipitation synthesized Ca_3SiO_5 (shown in Figures 4c-f) exhibit similar agglomerated structures, though with some distinct differences. The hydrothermal synthesis produced particles with relatively more uniform size distribution compared to the co-precipitation method, likely due to the controlled nucleation and growth conditions within the autoclave environment. The co-precipitation derived particles appear more irregular and heterogeneously sized, consistent with the less controlled precipitation kinetics. Despite these variations, all three synthesis methods produced fine agglomerated powders, which is typical for nanocrystalline ceramic materials. The observed agglomeration may influence the surface area and dissolution behavior of the materials, thereby affecting their biological performance.

3.4. Cumulative Ion Release Study

The ion release behavior of the synthesized Ca_3SiO_5 materials was investigated by immersing 0.1 g of each sample in phosphate-buffered saline (pH 7.4) at 37°C for up to 21 days. The cumulative release profiles of Ca^{2+} and Si^{4+} ions, determined by ICP-OES analysis, are presented in Figures 5(a) and 5(b), respectively.

The calcium ion release profiles (Figure 5a) reveal distinct differences among the three synthesis methods. Hydrothermally synthesized Ca_3SiO_5 exhibited the highest

cumulative Ca^{2+} release, reaching approximately 108 ppm after 21 days, followed by the co-precipitation derived material. In contrast, the sol-gel synthesized sample showed the lowest Ca^{2+} release, suggesting a more stable and less soluble structure. This observation correlates well with the lower lattice strain and higher crystallinity of the sol-gel material, which likely reduces its susceptibility to dissolution. Conversely, the higher Ca^{2+} release from hydrothermally and co-precipitation synthesized materials may be attributed to their greater lattice strain and associated structural defects, which facilitate ion exchange and dissolution processes.

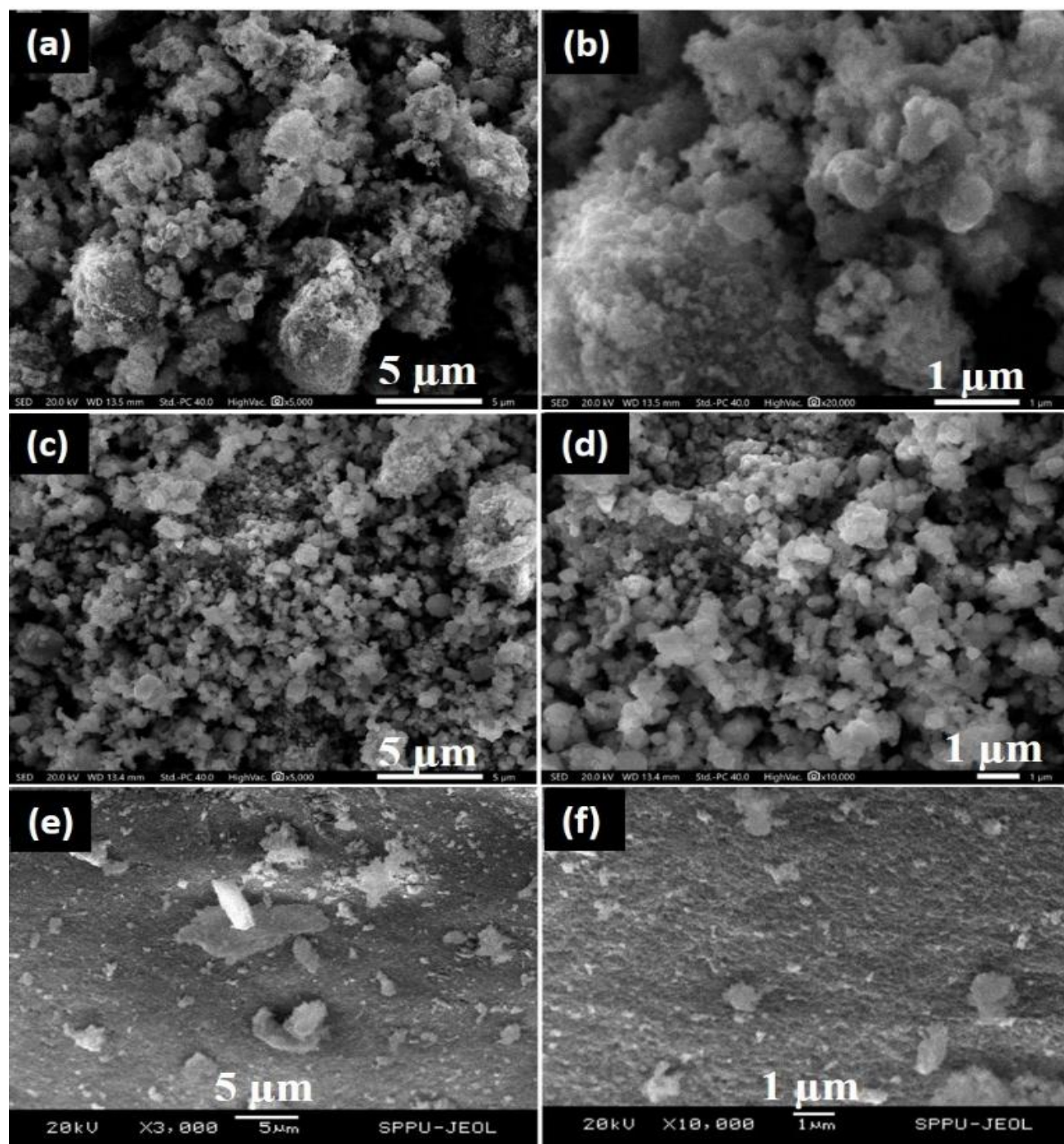


Fig. 4. SEM images of Ca_3SiO_5 synthesized by (a, b) sol-gel, (c, d) hydrothermal, and (e, f) co-precipitation methods.

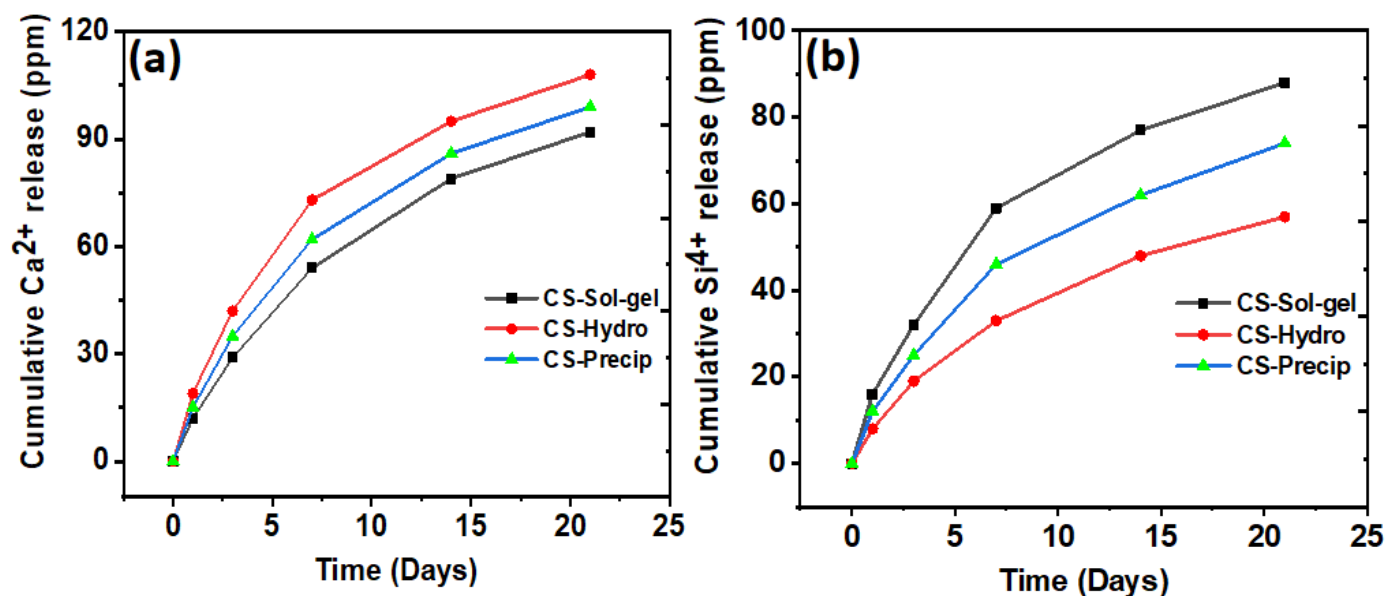


Fig. 5. Cumulative (a) Ca^{2+} and (b) Si^{4+} ion release profiles from Ca_3SiO_5 synthesized by different methods as a function of immersion time in PBS (pH=7.4, 37°C).

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The silicon ion release profiles (Figure 5b) showed an opposite trend, with the sol-gel derived Ca_3SiO_5 exhibiting the highest Si^{4+} release of approximately 88 ppm after 21 days, while hydrothermally synthesized material demonstrated the lowest silicon ion release. This inverse relationship between Ca^{2+} and Si^{4+} release suggests that the dissolution mechanism and ion exchange dynamics are influenced by the structural characteristics imparted by each synthesis method. The higher Si^{4+} release from sol-gel material is particularly noteworthy, as silicon ions are known to play a crucial role in stimulating osteoblast proliferation and differentiation. The controlled release of both Ca^{2+} and Si^{4+} ions from the sol-gel material appears to provide an optimal balance, potentially contributing to its superior biological performance.

3.5 Biological Response Evaluation

3.5.1. Cell Viability Assessment

The cytocompatibility of the synthesized Ca_3SiO_5 materials was evaluated using human osteoblast-like MG-63 cells through MTT assay [26, 27]. The cell viability results, expressed as percentage relative to untreated control cells, are presented in Figure 6(a) for concentrations ranging from 0 to 200 $\mu\text{g/mL}$ over a 72-hour culture period. All three materials demonstrated excellent cytocompatibility, maintaining cell viability above 95% across all tested concentrations, confirming their non-cytotoxic nature.

A distinctive peak in cell viability was observed at 50 $\mu\text{g/mL}$ for all three materials, indicating that this concentration optimally stimulates cellular metabolic activity and promotes proliferation. At this concentration, the sol-gel derived material showed the highest cell viability, followed by hydrothermal and co-precipitation samples. At higher concentrations ranging from 100 to 200 $\mu\text{g/mL}$, cell viability remained close to 100% relative to the control, demonstrating that the materials maintain excellent biocompatibility even at elevated doses. The MTT assay, which measures mitochondrial dehydrogenase activity as an indicator of metabolically active cells, confirmed that the reduction of yellow MTT to purple formazan crystals was most pronounced in cultures treated with sol-gel synthesized Ca_3SiO_5 [27]. These results collectively establish that all three synthesis methods produce biocompatible materials, with the sol-gel approach yielding the most favorable cellular response.

3.5.2. Alkaline Phosphatase Activity

The osteogenic differentiation potential of the synthesized materials was assessed by measuring alkaline phosphatase activity, an early marker of osteoblast maturation and bone

matrix formation [28, 29]. Figure 6(b) shows the ALP activity of MG-63 cells cultured with varying concentrations

of Ca_3SiO_5 for 72 hours, expressed as percentage relative to untreated control cells.

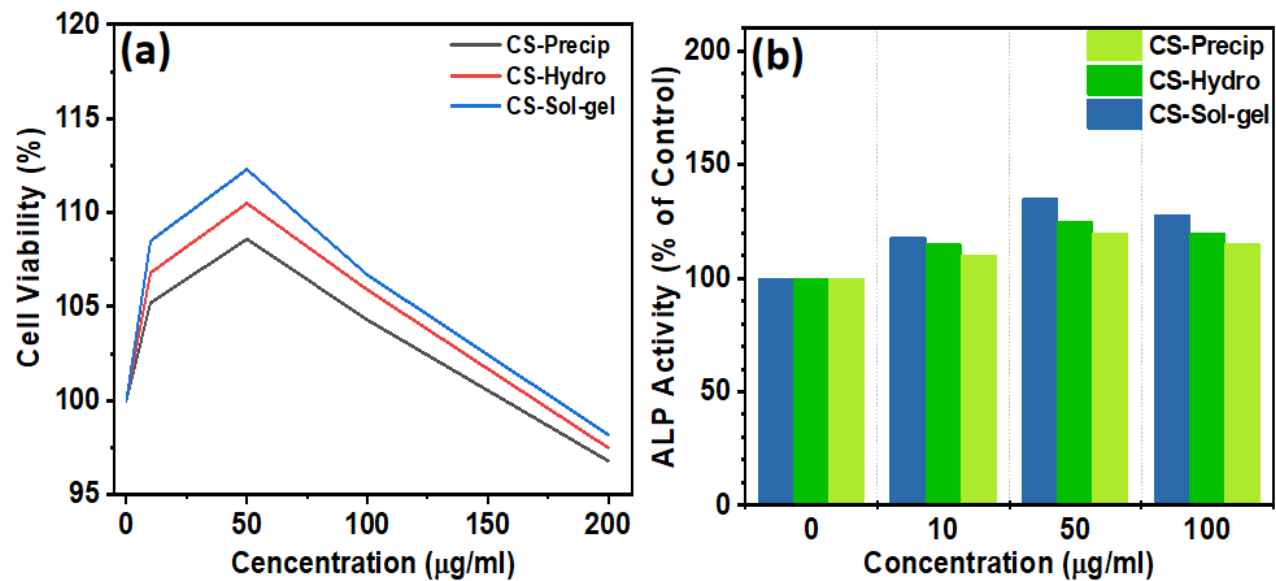


Fig. 6. (a) Cell viability (%) and (b) ALP activity (%) of MG-63 osteoblast-like cells cultured with Ca_3SiO_5 synthesized by sol-gel (CS-sol-gel), hydrothermal (CS-Hydro), and co-precipitation (CS-Precip) methods at various concentrations.

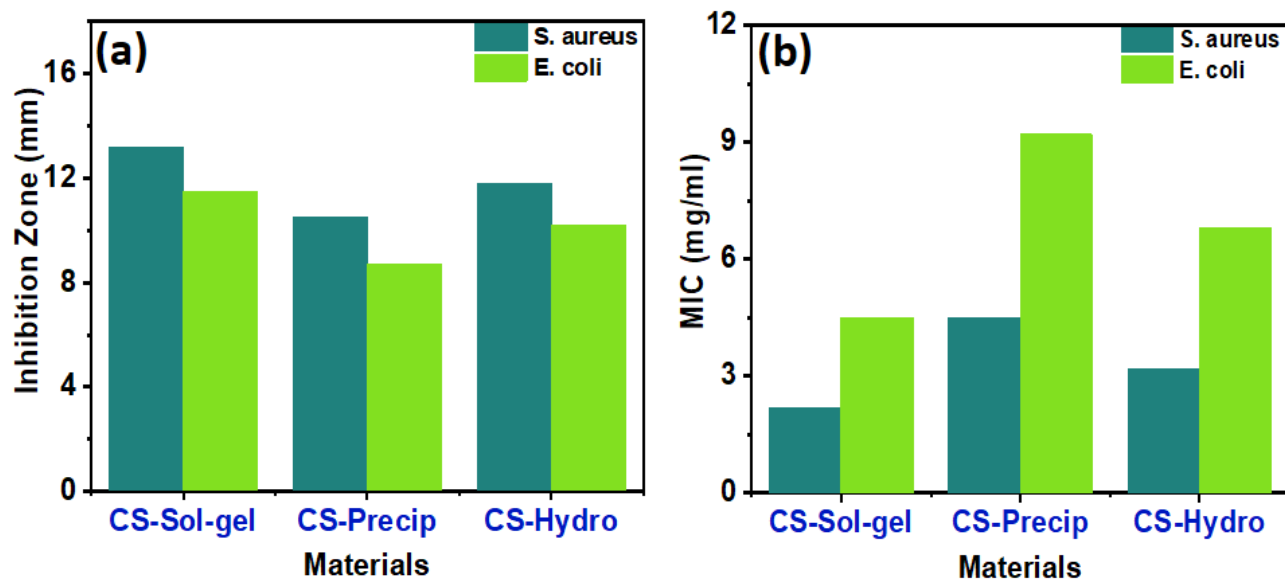


Fig. 7. (a) Zone of inhibition (mm) and (b) Minimum inhibitory concentration (µg/mL) of Ca_3SiO_5 synthesized by sol-gel, hydrothermal, and co-precipitation methods against *Staphylococcus aureus* and *Escherichia coli*.

Consistent with the cell viability results, maximum ALP activity was observed at 50 µg/mL for all three materials. Remarkably, all synthesized Ca_3SiO_5 samples exhibited significantly higher ALP activity than the control (100%) across concentrations ranging from 0 to 100 µg/mL, confirming their osteoinductive potential. Among the three synthesis methods, sol-gel derived Ca_3SiO_5 consistently outperformed the others, achieving the highest ALP activity

of approximately 135% relative to the control at 50 µg/mL. The hydrothermal and co-precipitation materials also demonstrated enhanced ALP activity, though at slightly lower levels, reaching approximately 125% and 120% of the control, respectively.

These findings indicate that Ca_3SiO_5 actively signals osteoblasts to initiate the bone-forming cascade, with the sol-gel material exhibiting superior osteogenic stimulation. The

enhanced ALP activity observed with sol-gel derived material can be attributed to its optimal ion release profile, particularly the higher Si^{4+} release, which is known to upregulate the expression of osteogenic genes and promote osteoblast differentiation. The lower lattice strain and higher structural ordering of the sol-gel material likely facilitate controlled ion release kinetics that are more favorable for cellular signaling pathways involved in osteogenesis.

3.6. Antibacterial Analysis

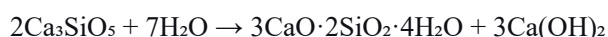
The antibacterial efficacy of the synthesized Ca_3SiO_5 materials was evaluated against Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922) using agar diffusion and minimum inhibitory concentration (MIC) assays [30, 31].

Figure 7(a) presents the zone of inhibition diameters for the three materials against both bacterial strains. The sol-gel synthesized Ca_3SiO_5 produced the largest inhibition zones, measuring approximately 13 mm against *S. aureus*, indicating its superior antibacterial activity. Hydrothermal and co-precipitation derived materials showed progressively smaller inhibition zones, ranking second and third, respectively. Across all materials, the inhibition zones were consistently larger against *S. aureus* compared to *E. coli*, suggesting that Gram-positive bacteria are more susceptible to the antibacterial mechanism of Ca_3SiO_5 . This differential sensitivity can be attributed to the structural differences between Gram-positive and Gram-negative bacteria, with the latter possessing an outer membrane that provides an additional barrier against external stressors.

The MIC values, representing the lowest concentration required to inhibit visible bacterial growth, are shown in Figure 7(b). Consistent with the zone of inhibition results, the sol-gel synthesized material exhibited the lowest MIC, confirming its superior antibacterial potency. The co-precipitation derived material required approximately twice the concentration of sol-gel material to achieve comparable antibacterial effect against *S. aureus*. These results demonstrate that sol-gel synthesized Ca_3SiO_5 is approximately twice as potent as the co-precipitation material, making it a highly attractive candidate for applications requiring both antibacterial protection and osteogenic stimulation.

3.7 Mechanism of Osteogenicity

The remarkable osteogenic potential of Ca_3SiO_5 arises primarily from its inherent bioactivity, which enables the formation of a biologically active hydroxyapatite layer on its surface and the controlled release of osteogenic signaling ions [14, 15]. When Ca_3SiO_5 comes into contact with physiological fluids, it undergoes hydration according to the following reaction.



This reaction releases Ca^{2+} and silicate ions (SiO_4^{4-}) into the local microenvironment, creating a chemically active interface. The released Ca^{2+} ions undergo exchange with H^+ ions from the surrounding medium, generating silanol-rich (Si-OH) groups on the material surface. These silanol groups, being negatively charged, attract Ca^{2+} ions from the physiological environment, creating a locally supersaturated calcium concentration that subsequently attracts phosphate ions (PO_4^{3-}). This sequence of events leads to the nucleation and growth of a hydroxyapatite-like layer on the material surface, which is essential for direct bone bonding and integration.

Beyond providing a substrate for hydroxyapatite formation, the released ions serve as chemical messengers that modulate cellular behavior. Silicon ions, in particular, have been shown to stimulate osteoblast proliferation and upregulate the expression of bone-related genes through the activation of specific signaling pathways. The elevated alkaline phosphatase activity observed in cells cultured with Ca_3SiO_5 , especially the sol-gel variant, confirms enhanced osteoblast differentiation. ALP catalyzes the hydrolysis of organic phosphate esters, increasing the local concentration of inorganic phosphate required for mineralization of the bone matrix. The superior performance of sol-gel derived material can be attributed to its optimal ion release kinetics, which provide sustained concentrations of bioactive ions necessary for efficient osteogenic signaling [5, 8].

3.8 Mechanism of Antibacterial Efficiency

The antibacterial activity of Ca_3SiO_5 against *S. aureus* and *E. coli* is predominantly mediated through the generation of "alkaline stress" during material degradation [6, 7]. Upon exposure to aqueous environments, Ca_3SiO_5 undergoes hydration, releasing calcium hydroxide that dissociates into Ca^{2+} and OH^- ions. This process rapidly elevates the local pH to highly alkaline levels, often exceeding 11, creating an environment that is hostile to bacterial survival.

The alkaline environment induces the generation of reactive oxygen species (ROS), which attack the polyunsaturated fatty acids present in bacterial cell membranes through lipid peroxidation. This oxidative damage compromises membrane integrity, leading to leakage of intracellular components including proteins, ions, and nucleic acids. Simultaneously, the high pH disrupts hydrogen bonding and ionic interactions that maintain the three-dimensional conformation of essential bacterial enzymes and transport proteins, rendering them non-functional.

In Gram-positive bacteria such as *S. aureus*, the absence of an outer membrane makes them particularly vulnerable to these alkaline and oxidative stresses. The hydroxyl ions penetrate more readily, causing direct damage to the cell wall and cytoplasmic membrane. In *E. coli*, the antibacterial mechanism also involves disruption of the proton motive force essential for ATP synthesis, as the abundance of OH^- ions neutralize protons required for maintaining the

electrochemical gradient across the inner membrane. This effectively short-circuits bacterial energy production, leading to metabolic collapse.

The alkaline environment also damages bacterial DNA by reacting with hydroxyl groups, inhibiting replication and protein synthesis. The combined effects of membrane damage, protein denaturation, enzyme inactivation, and DNA damage contribute to the potent antibacterial activity of Ca_3SiO_5 . The superior performance of sol-gel synthesized material can be attributed to its controlled ion release characteristics, which maintain sustained alkaline conditions favorable for antibacterial action while supporting osteogenic cellular responses [30-32].

4. CONCLUSION

This study systematically compared tricalcium silicate (Ca_3SiO_5) synthesized via sol-gel, hydrothermal, and co-precipitation methods to identify the optimal synthesis route for dual osteogenic and antibacterial applications. All three techniques yielded nanocrystalline materials with crystallite sizes around 10 nm; however, significant differences emerged in phase purity, structural perfection, and biological performance. The sol-gel and hydrothermal methods provided superior phase control compared to co-precipitation, which produced minor Ca_2SiO_4 impurities. Notably, sol-gel derived Ca_3SiO_5 exhibited the lowest lattice strain (0.1119), indicating a more ordered crystal structure with fewer defects. Biological evaluations demonstrated that sol-gel synthesized material consistently outperformed its counterparts, achieving the highest alkaline phosphatase activity (~135% of control) and exhibiting superior antibacterial efficacy with inhibition zones of approximately 13 mm against *Staphylococcus aureus* and the lowest minimum inhibitory concentration. These enhanced properties are attributed to its optimal ion release profile, particularly the highest Si^{4+} release (~88 ppm), and controlled degradation behavior that creates sustained alkaline conditions favorable for both osteogenic differentiation and bacterial inhibition. While hydrothermal and co-precipitation methods remain cost-effective alternatives, the sol-gel process provides superior balance of biocompatibility, osteogenic signaling, and antimicrobial protection. These findings establish sol-gel derived Ca_3SiO_5 as a highly promising bioactive material for treating complex bone defects where both regeneration and infection control are critical, while also providing fundamental insights into structure-property-activity relationships for designing next-generation multifunctional bioceramics.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under

consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

A.Z. Sayyed and A.M. Patil contributed equally to this work.

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